The adjuvant of the invention comprises the above-mentioned attenuated toxin alone or in combination with a preservative, stabilizer, and/or a conventional adjuvant. In general, the safety of an adjuvant is judged by repeatedly measuring safety. In addition, it is preferable to evaluate the safety by adopting a method using a living cell or living body as one of the indexes. As a matter of course, a conventional safety test, in which acute toxicity is examined by administering the adjuvant into experimental animals, should be conducted. Further, the absence of irritability on the skin and mucous membrane is an important safety index for oral administration, percutaneous administration and intranasal administration.

Adjuvant activity:

The present invention utilizes, as the adjuvant, an attenuated toxin in which the toxic activity is attenuated to at least one-two thousandth that of the natural toxin, as determined by a particular attenuation method, and in which the adjuvant activity is maintained. The adjuvant effect can be maintained at a high degree in the toxin by retaining the three types of amino acid residues that are specified in the summary above, namely serine, glutamic acid, and lysine. However, it is necessary to test whether or not the adjuvant activity is reduced during the process of treatment to reduce the toxic activity. The adjuvant activity can be verified by immunizing an animal with a substance to be compared for the activity, together with an immunological antigen, and then by observing the increase in titer of antibody against the immunological antigen. It is possible to compare the adjuvant activity by using a uniform condition like using animals of the same line and using identical immunological antigen.

Usage pattern:

There is no particular limitation on the usage pattern for the attenuated toxin of the present invention as an active ingredient in a vaccine. In other words, the adjuvant can be used with various known appropriate usage patterns. For example, the adjuvant may be a physically mixed preparation or a complex chemically linked with an antigen protein. In addition, the adjuvant can be incorporated

together with a vaccine in a carrier such as liposome.

The attenuated toxin of the invention can be used concurrently together with one or more conventional adjuvants. Example 5 shows the combined use of the attenuated cholera toxin adjuvant of the present invention with *E. coli* heat-labile toxin B subunit that is a conventionally known adjuvant. When used together with *E. coli* heat-labile toxin B subunit, the attenuated toxin adjuvant elevated the immunological activity in mucous membrane and further exhibited a synergistic effect. Those skilled in the art can readily find a preferable combination of adjuvants using routine experimentation, using publicly known methods. Based on the result obtained, it is possible to reduce adverse side reactions and enhance the immunoreactivity, for example, by reducing the amount of antigen or the other adjuvant.

Vaccine:

New vaccine preparations are provided by utilizing the adjuvants of the present invention. A vaccine preparation in the context of the present invention comprises the inventive adjuvant and any immunogenic antigen, and encompasses "vaccines" in both narrow and broad senses. Specifically, the vaccines of the present invention include vaccines in the narrow sense, such as those that are effective against infection by a virus, bacterium, fungus, protozoan, or other microorganisms capable of infecting to human or other animals. Illustrative examples of such vaccines include, but are not limited to, influenza vaccine, pertussis vaccine, purified pertussis-diphtheria-tetanus toxoids combined vaccine, Japanese encephalitis vaccine, hepatitis A vaccine, hepatitis B vaccine, rotavirus vaccine, measles vaccine, rubella vaccine, mumps vaccine, measles-rubella-mumps trivalent vaccine, measles-rubella divalent vaccine, vaccine against haemophilus influenzae, etc. The vaccines also include tuberculosis vaccine, vaccine against multi-drug resistant Staphylococcus aureus (MRSA), *Helicobacter pylori* vaccine, enterohaemorrhagic *Escherichia coli* (EHEC) vaccine, salmonella vaccine, *Chlamydia* vaccine, *Mycoplasma* vaccine, AIDS vaccine, malaria vaccine, coccidium vaccine, and schistosome vaccine. Vaccines in the

broad sense include those vaccines that are effective in the prevention and treatment of non-infectious diseases such as anti-cancer vaccine, vaccine of contraception, anti-gastric ulcer vaccine, anti-diabetic vaccine and anti-arteriosclerotic vaccine. Vaccines can be administered by injection, orally, percutaneously, intranasally or by other methods.

The vaccines of the present invention include various vaccines that can be categorized based on the types of methods to produce them. Specifically, the vaccines of the present invention include attenuated live vaccines, inactivated vaccines, component vaccines, vaccines using DNA, etc. The "vaccines using DNA" include both vaccines containing a DNA fragment integrated in a carrier, such as plasmid, and vaccines used in combination with ribozymes or antisense oligonucleotides, and the like. These vaccines can be used for prevention or treatment. The vaccines also include recombinant vaccines containing an antigen, which is the active ingredient of vaccine, produced in living cells engineered by gene recombination techniques. These vaccines may be monovalent vaccines or combined vaccines. Illustrative production methods and usage patterns of the vaccines are as follows.

- Influenza vaccine: a split vaccine which contains hemagglutinin (HA), neuraminidase (NA), nuclear protein (NP), matrix protein (M), or a part thereof, obtained by (a) growing viruses in embryonated eggs or in Vero cells or by other animal cell culture techniques and (b) decomposing the virus particles into respective components thereof with ether, detergent or the like and purifying the virus components, or by genetic engineering or chemical synthesis and which is administered by injection, orally, percutaneously or intranasally; alternatively, a DNA vaccine containing DNA fragments of genes encoding these proteins to be given by intranasal route.
- Pertussis vaccine: an inactivated vaccine that is obtained by culturing *Bordetella pertussis*, treating the culture supernatant or bacteria by salting-out, ultracentrifugation, and the like to extract constituents of interest, and detoxicating with formalin; alternatively, a recombinant vaccine containing *Bordetella*